

Maximizing Efficiency of Recurrent Phenotypic Selection for Neutral Detergent Fiber Concentration in Smooth Brome grass

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ABSTRACT

Resources are always limited in plant breeding programs, limiting the number of plots or plants that can be utilized in recurrent selection. Replication of selection units in space or time provides a mechanism to improve the precision of measurements. For smooth brome grass (*Bromus inermis* L.), neutral detergent fiber (NDF) is the most effective and measurable predictor of animal intake, a major source of variation for livestock production. The objective of this experiment was to determine the potential benefits and costs of replication in time and space for divergent recurrent selection on NDF of smooth brome grass. Phenotypic data was used to predict selection response and derive a benefit–cost ratio for a variety of selection criteria based on various numbers of blocks and harvests. Three of the 70 selection criteria examined had an expected selection response significantly greater than that predicted by a log-linear regression of expected selection response as a function of cost. These selection criteria were based on two or three harvests and one or two blocks. Two of the selection criteria, emphasizing one harvest of unreplicated plants, had a benefit–cost ratio significantly above the mean. If financial resources are not a concern, selection based on the mean of multiple harvests in 1 yr results in the highest expected response per year. If financial resources are greatly limited, one harvest of unreplicated plants provides an adequate expected response per cycle but maximizes gain per unit cost.

RECURRENT SELECTION is a breeding strategy that increases the frequency of favorable alleles of a specific trait in a population by eliminating the gametes of inferior parents from contributing to the next generation while maintaining genetic diversity (Comstock, 1996, pp. 69–72). This method has shown measurable selection gain for forage quality traits in reed canarygrass, *Phalaris arundinacea* L. (Surprenant et al., 1988); timothy, *Phleum pratense* L. (Claessens et al., 2004); and smooth brome grass (Casler, 2002).

One potential pitfall of recurrent selection is the possibility of inbreeding due to the restriction of population size. Divergent recurrent selection can be employed as a tool to separate the effects of inbreeding from true selection gain (Casler, 2002; Falconer, 1953). This method has been effective in creating populations divergent for fiber concentration in reed canarygrass (Surprenant et

al., 1988), timothy (Claessens et al., 2004), and smooth brome grass (Casler, 2002).

Resources are always limited in plant breeding programs, limiting the number of plots or plants that can be utilized in recurrent selection. Thus, it is imperative that phenotypic data is gathered in such a way to maximize the accuracy and precision of measurements. Replication of selection units in space and/or time provides a mechanism to improve the precision of measurements. Heritability can be increased by clonal propagation of selection units (England, 1977). There is always some advantage in replicating selection units if it can be accomplished without reducing selection intensity, especially when the trait of interest has low heritability (England, 1977).

In addition, taking measurements on several harvests throughout the year leads to more precise measurements as well as an increased likelihood of selecting truly superior plants (Aung et al., 1994). Five repeated measurements provides an 83% chance of selecting at least three of the five most extreme genotypes, a 52% chance of selecting at least four of the five most extreme genotypes, and a near certainty that the most extreme genotype will be identified (Aung et al., 1994). Utilizing repeated measures of body condition score improved the ability to predict fertility in dairy cattle by 28 to 53% compared with single-measure analysis (Banos et al., 2004). Taking this to an extreme can actually reduce the efficiency of selection both in terms of costs due to labor and in gain per year by increasing the amount of time spent collecting and analyzing multiple measurements on the same selection units, leading to decreased benefit per unit cost.

Neutral detergent fiber (NDF) is the most effective and measurable predictor of animal intake (Van Soest, 1994). About 70% of the variation in animal production is attributed to differences in intake potential of livestock feed (Crampton et al., 1960). Divergent selection for NDF concentration in smooth brome grass resulted in a direct response of 0.7 to 1.3% of population means (Casler, 2002). Genetic improvement for NDF has also been achieved in timothy (Claessens et al., 2004) and reed canarygrass (Surprenant et al., 1988). While gains were achieved in these studies by selecting on the basis of unreplicated plants harvested once (smooth brome grass and timothy) or twice (reed canarygrass), heritability for NDF was generally low. Casler (1999) reported the realized heritability for NDF concentration in smooth brome grass as 0.31. The objective of this experiment was to determine the potential benefits and costs of replication in time and space for divergent recurrent selection on NDF of smooth brome grass.

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MATERIALS AND METHODS

Germplasm and Pedigree

Divergent selection for neutral detergent fiber was performed on four populations of smooth brome grass. The four populations, Alpha, Lincoln, WB19e, and WB88S, were unrelated to each other except that the Wisconsin populations, Alpha and WB19e, shared approximately 25% of their alleles (Casler et al., 2000) and a population selected for high digestibility out of Lincoln was also one out of four parents of WB19e (Diaby and Casler, 2005). Lincoln is a land race cultivar created from a wild collection from Hungary and WB88S is a Syn1 strain-cross created from five accessions collected from the Altai Mtns. of southern Russia (USDA-ARS, 1990).

The first cycle of divergent selection for NDF was completed as described by Casler (2002) creating low NDF (C-1) and high NDF (C+1) subpopulations by polycrossing the 10 plants with the highest or lowest NDF values selected out of 300 spaced-plants from each population on the basis of data from one harvest of leaf tissue. Diaby and Casler (2005) conducted the second cycle of divergent selection for NDF creating C-2 and C+2 subpopulations for each of the four populations using the same method. Plants from the original germplasm were designated as C0, resulting in five subpopulations for each population. A total of 370 plants, representing a random sample of 12 to 26 seedlings from each of these 20 subpopulations, were grown and maintained in a greenhouse. Plants that were sufficiently large were clonally propagated between December 2001 and May 2002.

Field Evaluation

All plants were transplanted into the field in a spaced-plant nursery on 0.9-m centers in June 2002 at Arlington, WI. The experimental design was a randomized complete block design with two clonal replicates. Within each replicate, clones were blocked according to a split-plot randomization restriction, with populations as whole plots and clones as sub-plots. Plants were clipped twice during the establishment year, but no samples were collected. Weeds were controlled by a combination of tillage, hand weeding, and application of 1.12 kg ha⁻¹ alachlor [2-chloro-*N*-2,6-diethylphenyl]-*N*-(methoxymethyl)-acetamide] with 0.07 kg ha⁻¹ imazethapyr [(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid]. Plants were fertilized with 56 kg N ha⁻¹ in early spring 2003, early spring 2004, and following every clipping.

Starting in 2003, leaf blades and sheaths were harvested from each plant. Leaf blades and sheaths were meant to represent a pasture-based management system. Harvests were taken in May, June, August, and October in 2003 and 2004, when the canopy height ranged from 20 to 30 cm. Plants were hand-clipped, leaving a stubble height of 9 cm. The tissue samples were collected in paper bags and dried at 60°C. Dried samples were ground through a 1-mm screen with a Wiley-type mill. All samples were scanned by near-infrared reflectance spectroscopy (NIRS). Subsets of 80 samples from each year were selected by cluster analysis of wavelength reflectance data (Shenk and Westerhaus, 1991). These samples were analyzed for NDF concentration by using the procedure outlined in Van Soest et al. (1991), omitting the α-amylase step. The NDF values were predicted for all samples by two calibration equations: SE_{cal} = 12.8 g kg⁻¹, R_{cal}² = 0.93, SE_{val} = 17.7 g kg⁻¹, and R_{val}² = 0.87 for 2003 data and SE_{cal} = 10.8 g kg⁻¹, R_{cal}² = 0.95, SE_{val} = 14.9 g kg⁻¹, and R_{val}² = 0.90 for 2004 data.

Statistical Analyses

Because of numerous missing plants, occasional plant mortality, and many samples of insufficient size for analysis, samples were retained from only 150 clones that were completely balanced across both replicates and the last seven harvests (excluding the May 2003 harvest). Between five and 12 plants were included from each of the 20 subpopulations described earlier. The NDF values were analyzed by generalized least squares analysis of variance assuming all effects to be fixed except replicates. Normality of the distribution of genotype means was tested by the Shapiro-Wilk test (SAS, 1999).

A total of 105 potential selection criteria were created as a combination of 35 repeated-measures criteria with three replication criteria (Block 1, Block 2, or both blocks). One of these, based on both blocks and all seven harvests, was defined as the best available measure of NDF. The 35 repeated-measures criteria involved various combinations of harvests ranging from one to seven harvests. For each of the 105 selection criteria, genotypes were ranked for mean NDF defined by that particular criterion. For each criterion, the five highest-ranked and the five lowest-ranked genotypes were chosen. The selection differential for each criterion was then computed as the difference between means of the divergent groups of genotypes, identified by the selection criterion, but using data from means over both blocks and all seven harvests. Thus, the selection differentials themselves accounted for the genetic correlation between the selection criterion and the best available measure of NDF, with genotypes chosen by one criterion and selection differentials computed from the best available measure of NDF. This was done partly for simplicity and partly because it was impossible to reliably estimate the genetic correlation between a selection criterion and the best measure of NDF, because of the necessarily incomplete data structure and the fact that each selection criterion is part of the identity of the best measure of NDF. For one of the 105 selection criteria ($b = 2$ and $h = 7$), this selection differential was defined as the best measure of NDF, making this value a direct measure of selection differential, rather than an indirect measure as for the other 104 selection criteria.

Expected responses (R_i) for each selection criterion (i) were calculated by the equation

$$R_i = SD_i \times h_i^2 \times y_i^{-1}$$

where SD_i is the selection differential, h_i^2 is heritability, and y_i is the number of years per cycle ($y = 3$ if only harvests from 2003 were considered or $y = 4$ if any 2004 harvests were used) for the i th selection criterion. A 3-yr phenotypic selection scheme was defined as follows: Year 1 – establish selection nursery, Year 2 – harvest samples and conduct laboratory analyses, and Year 3 – establish crossing blocks and harvest seed. The h_i^2 values used to predict response were synthesized as

$$h_i^2 = s_A^2 / [s_A^2 + s_{GB}^2/b_i + s_{GH}^2/c_i + s_A^2/(b_i c_i)]$$

where b_i is the number of replicates and c_i is the number of harvests involved in the i th selection criterion; s_{GB}^2 , s_{GH}^2 , and s_e^2 are the estimated variance components for genotype × block interaction, genotype × harvest interaction, and error, respectively; and s_A^2 is the estimate of additive genetic variance. The variance components s_{GB}^2 , s_{GH}^2 , and s_e^2 were estimated by equating mean squares from the NDF analysis of variance to their expectations (Gaylor et al., 1970). The estimate of additive genetic variance was computed by entering values of s_{GB}^2 , s_{GH}^2 , and s_e^2 into the equation for h_i^2 , setting $b_i = 1$ and $c_i = 1$, setting $h_i^2 = 0.31$, based on realized heritability estimates of Casler (1999) for selection unreplicated in space or

Table 1. Generalized least squares analysis of variance for neutral detergent fiber (NDF) of 150 smooth brome grass genotypes evaluated over two blocks and seven harvests in 2003 and 2004.

Source of variation	df	Mean square	F value	P value
Harvest (H)	6	320 333	36.26	0.0002
Block (B)	1	27 081	3.07	0.1306
B × H	6	8 835	34.75	<0.0001
Genotype (G)	149	3 903	12.73	<0.0001
G × B	149	307	1.21	0.0597
G × H	894	476	1.87	<0.0001
Error	894	254		

time ($b = 1$ and $c = 1$), and solving for s_{α}^2 . This realized heritability estimate was derived from a different population, but represents the most reliable estimate for this selection criterion, method, and unit. It is consistent with other estimates of realized heritability for NDF, on the basis of the four populations included in the current study ($h^2 = 0.22$ – 0.34 ; our computations based on Casler, 2002).

Because the blocks served only as a form of replication in this study, expected response values for identical harvest selection criteria were averaged for Blocks 1 and 2, reducing the total number of selection criteria to 70. The 70 selection criteria were arranged into four groups on the basis of the number of blocks and years ($b = 1, y = 3; b = 1, y = 4; b = 2, y = 3; b = 2, y = 4$). Linear regression analyses were performed on the selection differentials against the number of harvests to quantify increases in selection differential as a function of number of harvests.

Two methods were used to objectively identify selection criteria with the greatest potential to improve the efficiency of selection for divergent NDF. First, the expected response from each of the 70 selection criteria was regressed against the relative cost of phenotyping using a log-linear model suggested by a plot of the data (Ratkowsky, 1990). The cost of phenotyping was defined as one arbitrary unit per harvest or block (ranging from 1–14), because the amount of time and labor involved in preparing the samples for analysis is proportional to the number of samples harvested and there was an equal number of plants in each block. An analysis of the residuals was used to identify selection criteria that produced an expected response significantly greater than that predicted by the log-linear regression model. Second, expected response was divided by cost to compute the benefit–cost ratio of each selection criterion. Superior selection criteria for benefit–cost ratio were identified as those with a ratio greater than two standard deviation units above the mean.

RESULTS AND DISCUSSION

The mean square for harvests was the largest in the analysis of variance, indicating large differences among harvests for mean NDF (Table 1). Thus, the inferences from this study span a relatively wide range in mean NDF values for the seven harvests (403–501 g kg⁻¹). Genotype and genotype × harvest interaction effects were both significant ($P < 0.0001$), but the mean square for genotype was much larger than that for genotype × harvest interaction. Therefore, the genotype × harvest interaction had relatively little biological significance in comparison to the variation between genotypes. This result was largely expected because numerous previous experiments have similarly shown genotype × environment interactions to be relatively unimportant for NDF of smooth brome grass (Reich and Casler, 1985; Casler and Vogel, 1999; Casler, 2002). Similarly, a previous

study of alfalfa (*Medicago sativa* L.) revealed no interactions between growth period or sampling times and cultivars for forage quality traits (Hall et al., 2000).

The phenotypic correlation structure was highly uniform among harvests, showing no patterns relative to year or time of year, with an average phenotypic correlation between harvests of $r = 0.51 \pm 0.03$. The phenotypic standard deviation among clone means was fairly constant across harvests, ranging from 16.7 to 24.8 g kg⁻¹. The rank correlation among harvests averaged $r_s = 0.50 \pm 0.03$, indicating that much of genotype × harvest interaction was due to changes in ranking of genotypes, not to changes in phenotypic variance. Genotype means for NDF were normally distributed within each harvest date and across harvests, effectively simulating origin from one source population.

Because selection differentials were always computed from means over both blocks and seven harvests, the J1-A1-O1-M2-J2-A2-O2 selection criterion based on both blocks had the highest value in Table 2. Twenty-one of the 35 selection criteria based on two blocks had a higher selection differential than their respective selection differential based on either individual block. Thirty-two of the 35 selection criteria based on two blocks had a selection differential higher than the mean of the selection differentials for their two respective one-block selection criteria. Each of the remaining three selection criteria, for which two blocks did not lead to a higher selection differential, was based solely or partly on the October 2003 harvest (O1, A1-O1, and O1-O2). Because the simple correlation between clonal observations in different blocks was highly uniform across harvests ($r = 0.52$ – 0.63 ; $P < 0.0001$), this observation could not be attributed to a differential correlation structure for the October 2003 harvest and its cause remains unknown.

Selection differentials generally increased as more harvests were included in the selection criterion, by 3.1 ± 0.5 and 2.7 ± 0.5 g kg⁻¹ harvest⁻¹ for one-block and two-block selection criteria, respectively (Table 2). This was a result of more accurate genotype rankings because of averaging over multiple repeated measurements of the phenotype (Aung et al., 1994). On average, spatial replication (mean NDF over two blocks vs. NDF value for one block) increased the selection differential by 3.4 ± 0.5 g kg⁻¹ block⁻¹, indicating that the effects of spatial and temporal replication were similar and interchangeable.

If the five clones with the highest or lowest NDF, based on means over two blocks and seven harvests, are defined as the “correct” selections, then the remaining selection criteria can be assessed for their ability to select these clones. Numbers of “correct” selections ranged from 3 to 9, based on means over two blocks (Table 2). The number of “correct” selections increased by 0.83 ± 0.14 ($P < 0.01$) for each additional harvest. Three selection criteria were notable, due a relatively high number of “correct” selections within their cost class (number of blocks × number of harvests): J2, J2-O2, and J2-A2-O2. However, the superiority of these three criteria were based on their exceptional ability

Table 2. Neutral detergent fiber (NDF) selection differentials and numbers of “correct” selections for various selection criteria based on data from various harvests in Block 1, Block 2, or as the mean over blocks.

Harvests†	Selection differential			Number of “correct” selections‡		
	Block 1	Block 2	Both blocks	High	Low	Total
	g kg ⁻¹					
J1	43	43	52	2	2	4
A1	38	47	51	3	2	5
O1	33	35	34	2	2	4
J1-A1	49	47	53	4	2	6
J1-O1	56	45	59	3	2	5
A1-O1	51	47	48	2	5	7
J1-A1-O1	58	51	60	4	3	7
M2	45	48	51	1	2	3
J2	52	47	54	5	2	7
A2	34	42	47	1	2	3
O2	49	51	54	2	3	5
M2-J2	54	57	56	4	2	6
M2-A2	54	50	57	2	2	4
M2-O2	53	59	59	3	2	5
J2-A2	57	50	59	4	3	7
J2-O2	59	55	59	5	3	8
A2-O2	50	52	53	2	3	5
M2-J2-A2	57	58	60	4	2	6
M2-J2-O2	59	61	61	4	2	6
M2-A2-O2	53	59	62	1	2	3
J2-A2-O2	60	57	62	5	3	8
M2-J2-A2-O2	60	62	62	4	3	7
J1-J2	52	53	55	3	2	5
J1-A2	55	52	54	4	3	7
A1-A2	52	52	56	2	3	5
O1-O2	51	48	48	2	3	5
J1-M2-J2	55	57	56	4	3	7
J1-A1-J2-A2	58	60	60	4	3	7
J1-O1-J2-O2	47	60	61	5	3	8
A1-O1-A2-O2	56	47	56	1	4	5
J1-A1-O1-M2-J2	58	62	61	4	4	8
J1-A1-M2-J2-A2	56	62	62	5	4	9
J1-A1-O1-M2-J2-A2	59	63	64	5	4	9
J1-A1-O1-J2-A2-O2	61	62	64	5	3	8
J1-A1-O1-M2-J2-A2-O2	61	62	64	5	5	10

† J1 = June of first year, A1 = August of first year, O1 = October of first year, M2 = May of second year, J2 = June of second year, A2 = August of second year, and O2 = October of second year.

‡ Number of clones identified as the five highest or lowest in NDF by the J1-A1-O1-M2-J2-A2-O2 selection criterion (the last line of the table), all based on means over two blocks.

identify high-NDF clones in particular; they did not differ from the remaining criteria in their ability to identify low-NDF clones. These three selection criteria are not considered desirable, because they are based entirely on second-year harvests, severely increasing cycle time and reducing gain per year. This criterion was not particularly useful in separating the remaining selection criteria within cost classes.

The natural logarithm of expected selection response was a linear function of relative cost (Fig. 1), in agreement with the genotype–replication tradeoff curve described by Gauch and Zobel (1996). Three of the 70 selection criteria had a significant positive residual value ($P < 0.05$), indicating an expected selection response greater than that predicted by the regression model. Replication in time or combined replication in space and time resulted in a sufficient increase in heritability to elevate these three selection criteria above those based on one block and one harvest (selection without any form of replication). These three selection criteria all had large selection differentials, high heritability, large predicted gains, and moderate benefit:cost ratios (2.2 to 3.4, relative to a mean of 2.4) (Table 3).

The benefit–cost ratio was approximately normally distributed (Fig. 2). Two selection criteria had a ratio

significantly above ($P < 0.05$) that of the mean ratio. These two selection criteria involved only one harvest in the first year. Both of these selection criteria had a lower expected response ($4.4\text{--}4.5\text{ g kg}^{-1}$) than the three selection criteria chosen on the basis of the log-linear regression residuals (Table 3). Benefit–cost ratios were greatly affected by relative cost, generally favoring selection criteria with lower cost and reduced expected gain (Fig. 2). Increasing either replication factor, blocks, or harvests, drastically decreased the benefit–cost ratio, particularly when the increased number of harvests resulted in the use of a second evaluation year ($y = 4$ in Fig. 2). A study of several sugarcane traits also demonstrated that eliminating the second year of phenotype analysis would benefit the efficiency of the breeding program (Brown and Glaz, 2001). Previous studies also indicate that decreasing the number of blocks results in higher response per unit cost (Osorio et al., 2003; Brown and Glaz, 2001). In both of these studies, the authors concluded that resources previously allocated to excessive replication would be utilized more efficiently by analyzing more genotypes or additional traits.

The most efficient method of phenotypic recurrent selection is highly dependent on the circumstances and needs of the breeding program. Harvesting two blocks

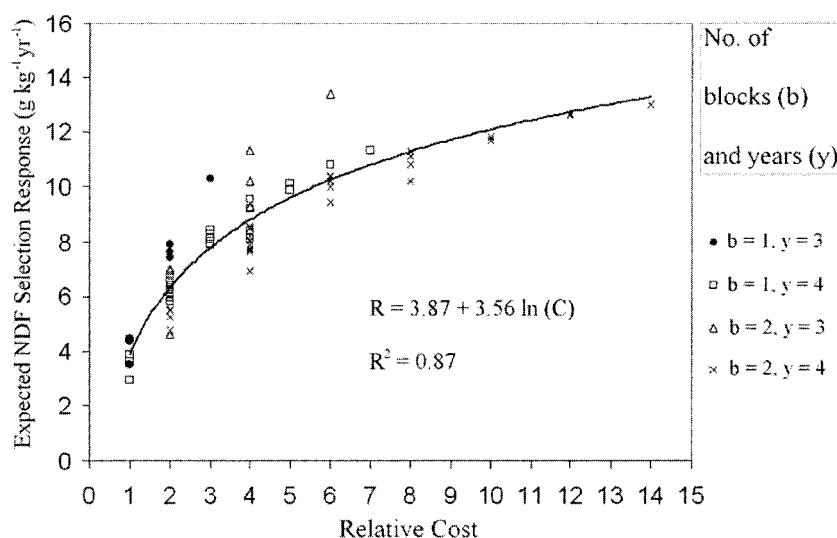


Fig. 1. Expected selection response (R) of neutral detergent fiber (NDF) concentration generated from 70 different selection criteria which were based on various numbers of blocks and harvests, regressed on the relative cost (C) of each selection criterion.

Table 3. Statistics of five selection criteria identified as having an expected response or benefit:cost ratio significantly higher than expected.

Harvests†	No. of blocks	Relative cost	Selection differential $g\ kg^{-1}$	h^2	Expected response‡ $g\ kg^{-1}\ yr^{-1}$	Ratio§
J1	1	1	43	0.31	4.5	4.5
A1	1	1	42	0.31	4.4	4.4
J1-A1-O1	1	3	55	0.56	10.3	3.4
J1-O1	2	4	59	0.58	11.3	2.8
J1-A1-O1	2	6	60	0.67	13.4	2.2

† J1 = June of first year, A1 = August of first year, O1 = October of first year.

‡ Selection criteria J1-A1-O1 (one block), J1-O1 (two blocks), and J1-A1-O1 (two blocks) were chosen using the residuals of the log-linear regression of this criterion.

§ Ratio = expected selection response divided by relative cost. Selection criteria J1

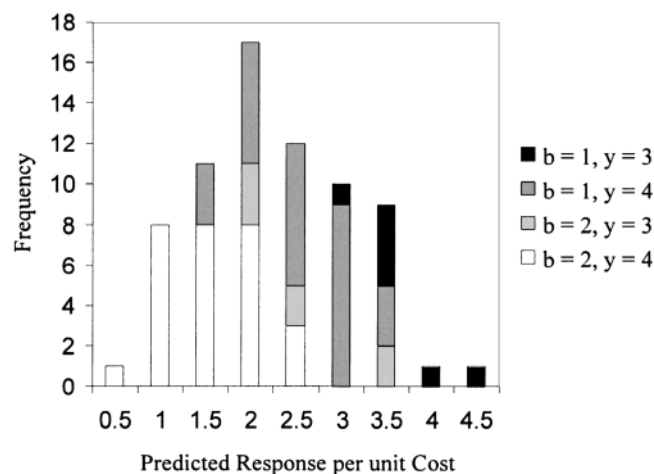


Fig. 2. Frequency distribution of the benefit-cost ratio for 70 selection criteria based on different numbers of blocks (b) or years per selection cycle (y).

in June, August, and October of the first year gave the largest predicted response, expressed on a gain-per-year basis. However, if financial resources do not permit such an investment, a reduction in either the number of harvests or blocks should maintain a relatively high selection response. These results demonstrated that the most effective response to phenotypic selection occurred when selection units were replicated using clonal propagules, in agreement with the results of England (1977).

The J1-A1-O1 selection criterion based on two blocks required six observations per genotype, which was close to the theoretical calculations of Gauch and Zobel (1996). For a signal-to-noise ratio of 0.65 (ratio of additive genetic variance to error variance using our data and formulas of Gauch and Zobel, 1996), a population size of 150 genotypes would require four replicates to maximize the expected true phenotype of the top selection. Because replication in either space or time was similarly effective for NDF selection of smooth brome-grass, multiple harvests and multiple blocks appear to be interchangeable. Because multiple harvests require less expense, replication in time may be preferable to replication in space, provided that samples are taken only within the first year. Selection gains may be greater for larger population sizes of unreplicated plants, but the increase in noise (random error effects) as replication is reduced or eliminated may negate the advantages of larger population sizes and larger selection differentials (Gauch and Zobel, 1996).

Harvesting only one block in June, August, and October of the first year provided a balance between the objectives of obtaining a high selection response and a high benefit-cost ratio. Furthermore, this selection criterion does not carry the additional cost of having to clonally replicate plants in the selection nursery, an additional expense of replication in space. Multiple harvests within the first year of nursery evaluation can

significantly improve the expected selection response for NDF, expressed on an absolute basis or per unit cost.

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